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New Approaches to Rose Breeding – The Arisumi Papers

M. S. Viraraghavan

During the period 1964-1968, the eminent Japanese biochemist, Dr. Kanichi Arisumi, published a series of four papers entitled 'Studies in the Flower Colours in Rosa' with special reference to biochemical and genetic analysis, and the application of these results to practical breeding. These papers, which represent a masterly synthesis of the subtleties of biochemistry and their application to rose breeding have remained relatively unknown to rosarians, though their content and importance is such that they deserve to be a part of their essential knowledge. This article is an attempt to present from a layman's point of view the main features of these papers, their significance to practical rose-breeding and some further speculations on the theme.

Before dealing with the papers, it is necessary to briefly discuss the various pigments which are responsible for the colours of flowers. All red, orange and blue flowers generally derive their colour from water soluble pigments occurring in the aqueous cell sap, called ANTHOCYANIDINS. The three important anthocyanidins are:

1. PELARGONIDIN - present in orange red to scarlet flowers
e.g. scarlet pelargonium (geranium),
orange red dahlia or vermillion roses like
'Super Star'.
2. CYANIDIN - present in crimson to bluish red flowers,
e.g. deep red dahlia, red roses such as
'Papa Meiland'.
3. DELPHINIDIN - present in violet to blue flowers such as
delphinium.

Apart from these three basic anthocyanidins there are certain other derivatives which are responsible for modified flower colour: in other words, various shades of red, vermillion, and blue. A number of other factors also influence the colour of anthocyanidins, e.g. iron salts intensify the colour, the pH of the cell sap, presence of tannins, organic acids and enzymes also play a part.

Other than anthocyanidins, there are a number of different pigments, such as FLAVANOLS (e.g. the colouring matter of the pale yellow China Rose 'Mme. Falcot'), and carotenoid pigments (the colouring matter in

carrots, or dark yellow roses), which are responsible for the white and yellow to brown range of flower colours. These pigments may be co-existent along with anthocyanidins with a consequent modification of the pure anthocyanidin colour range. It should also be noted that in many flowers the colour changes with the age of the bloom e.g. the colour change from yellow to red of the rose variety 'Charleston', and this is due to the modification of pigment composition during the ageing process.

In the light of the above, we may analyse the colours of roses as follows: Prior to 1900 there were only red (including pink), white and light yellow roses. The red and pink roses, e.g. the Hybrid Perpetuals, owed their colour to the presence of cyaniding; while the pale yellow colours, e.g. of the Tea roses were due to the presence of various types of flavonols. Initially the Hybrid Tea roses produced by crossing the Hybrid perpetual and the Teas had also the same colour range. But in the early years of the century (20th) the famous French hybridiser, Pernet Ducher, succeeded in crossing the then existing rose varieties into the bright yellow rose species *R. foetida* (Persian Yellow); thereby the colour range of roses was greatly increased, because Pernet Ducher's cross brought in the genes for the production of the carotenoid type of pigment. All the bright yellow roses of today ultimately owe their colour to the original cross made by him.

A second major landmark in the development of rose colour was the introduction of genes for the production of pelargonidin in roses. This occurred around 1930 and all today's vermillion or scarlet roses e.g. 'Super Star', owe their colour to pelargonidin.

As such we can see that the colour of modern roses is derived from CYANIDIN (red) and PELARGONIDIN (vermillion) as modified by the presence of various types of FLAVONOLS (pale yellow) and CAROTENOID pigments (dark yellow).

But prior to Arisumi's work it was believed that it was not possible to have a blue rose based on the pigment DELPHINIDIN, as this pigment was not present in any of the rose species. One of the most sensational aspects of Arisumi's work is the discovery for the first time of delphinidin in a rose. But I will deal with this in greater detail later.

The standard procedure adopted by Arisumi in his researches was to extract the pigments in various rose species, as well as in varieties belonging to different groups e.g. Teas, Hybrid Perpetuals, Gallica roses etc. and to separate the pigments into different types adopting the method of paper chromatography. The pigments present were identified; thereafter deductions were made on the genetic composition of the

various classes of roses in the light of pigment composition. Without going into too much detail the analysis carried out by Arisumi showed that the pigment in species roses was Cyanidin. But there were quite a few rose species containing other anthocyanidins such as PAEONIDIN and the modified cyaniding, CHRYSANTHAMIN. Most rose species also contained flavonols which were broadly divided into the KAEMPFEROL and QUERCETIN types. It is interesting to note that so many rose species contain paeonidin including the bicolour variant of *R. foetida*, *R. pomifera*, *R. californica*, *R. nitida*, *R. rugosa* and *R. stellata* variety *mirifica* in which significantly it is the only anthocyanidin present.

The analysis of pigments in garden roses showed a remarkable difference in the anthocyanidin constitution in Hybrid Teas and Floribundas, even though both are derived from the common ancestor species, *R. chinensis*. While the Floribundas had retained the original *chinensis*, i.e. Tea rose genes more or less intact, the Hybrid Teas, though they are the result of crosses between Hybrid perpetual and Tea roses, had largely the genetic composition of the Hybrid Perpetual – in other words, that of the tetraploid ancestral types such as Damask and Gallica roses, natives generally of the continent of Europe. As readers may have noted, one of the most interesting features of the *Chinensis* type of rose is the colour changing characteristics - originally of *R. chinensis mutabilis* and of which there are numerous examples in the Floribunda class, starting from 'Masquerade' and including 'Charleston' and many others.

The analysis of this pigment change showed that the colour variation occurred on account of the synthesis of a modified anthocyanidin (technically, formation of 3-monoside type anthocyanidin) as the flower developed from bud stage to the fully open stage. This feature was, at the time of the papers, confined to the Floribunda class, and Arisumi has pointed out that this is one more confirmation of the Floribundas containing the *Chinensis* type of gene which was lacking in the H.T.s.

Other major differences which Arisumi noted were the relative absence of pelargonidin in the Hybrid Tea class whereas a large percentage of the Floribundas contained pelargonidin. Again, many of the H.T.s studied had carotenoid type of pigments though the occurrence of carotenoid was much less frequent in Floribundas. In other words, Arisumi's conclusions support the common observation (again, more valid during the 1960s when his papers were written) that there are many more vermilion coloured Floribundas than H.T.s, but on the other hand, very few yellow Floribundas. In fact till today, the yellow Floribunda is a comparatively weak class.

So far we have dealt with the general features of Arisumi's work. Four very important propositions of great interest to rosarians and particularly rose breeders arise from his work, and I will deal with these individually below.

1. How do we evolve a rose which holds its colour without fading from the bud stage to the fully open stage?

Arisumi points out that the answer to this question lies in selective breeding with a view to produce such H.T.s which contain the genes for the formation of 3-monoside type anthocyanidin – both of the cyanidin type also the hitherto unknown and much brighter pelargonidin type (callistephin). As noted earlier, this ability originating from *R. chinensis mutabilis*, is already present in the Floribunda class. Arisumi had predicted in 1964 itself that if this feature could be transmitted to the H.T. class we would have very interesting results. Rosarians will note that it is only in the last few years, that is, well over a decade after Arisumi made the suggestion, that such roses have appeared in the H.T. class. I am referring to roses like 'Spellbinder' (Warriner 1975) which changes in colour from ivory to pale pink to almost crimson at the fully open stage; and the varieties 'Colour magic' (Warriner 1978) and 'Double Delight' (Swim & Ellis 1977). While 'Double Delight' changes from creamy white at bud stage to strawberry red, American commentators have remarked that 'Colour Magic', while changing colour basically from white to dark pink, changes in colour almost every hour after the bud has opened!

Doubtless, we will have even more interesting results when H.T.s are produced which change from yellow to red rather than from white to dark pink, such as happens in the Floribunda 'Charleston'. Much more exciting and spectacular would be H.T. where Callistephen is synthesized i.e. 3-monoside of the pelargonidin type.

Colour changing apart, I feel that the capacity to synthesize 3-monoside type anthocyanidin could be harnessed to produce a non-fading red H.T. which has long been the dream of rosarians. Attempts in this direction by introducing pelargonidin to boost the colour stability of the cyaniding red or by inter-crosses with yellow roses generally lead to beautiful red H.T.s completely lacking in the scent which rosarians rightly expect from a rose of that colour; for example, 'Christian Dior' or 'Samurai'. On the other hand, dark red H.T.s with fragrance generally tend to fade or 'blue' as they age - apart from usually being addicted to mildew.

A second approach to non-fading red colour is also suggested by the Arisumi papers, which I will deal with later.

2. Is it possible to produce a rose where the bright vermillion of pelargonidin and the dark yellow of the carotenoid type of pigments are together present to produce extraordinary brilliance of colour?

This is the famous proposition which is referred to by Dr. B. P. Pal in his well known work, *The Rose in India*, and which, in fact, started my search for the Arisumi papers. Arisumi's conclusion is that it is quite feasible to produce roses of such a type and he predicts in his papers that such roses will have a brilliance of colour not known in roses till then. I should again remind readers that this was a statement made around 1964. Thereafter such roses appeared in which the brilliance of pelargonidin is greatly intensified by the presence of carotenoid (my speculation!). I refer to roses like the H.T. 'Whisky Mac' or the incomparable Floribunda 'Belinda' – a pure golden vermillion, which are examples of the kind of achievement realized in recent years, envisaged so far back as Arisumi did.

But even more important than the results achieved so far is the guideline provided by Arisumi's work on how to achieve such a result. He points out that pelargonidin tends to co-exist with the Kaempferol type of yellow rose but is not normally found with the Quercetin kind. As mentioned earlier, Kaempferol and Quercetin are flavonols present in a number of rose species and varieties and generally found in most yellow roses in combination with the carotenoid type of pigment. Arisumi showed that while roses of the type of 'McGredy's Yellow' (1933) belong to the Kaempferol type, others like 'Lydia' (Robinson 1949) belong firmly to the Quercetin category. Crosses between 'McGredy's Yellow' and various vermillion roses carrying the pelargonidin gene resulted in a high proportion of the seedlings containing pelargonidin. Conversely, similar crosses with 'Lydia' led to the elimination of pelargonidin in the resultant seedlings.

Apart from the typical Quercetin and Kaempferol type of yellow roses there are others which fall into an intermediate category. Arisumi refers to the variety 'E. J. Baldwin' (Robinson 1952) in this connection. Here again his work is a little dated by now. But it is easy enough to deduce which of the present day yellow roses belong to the Quercetin or Kaempferol type, by referring to the parentage of modern vermillion roses. Clearly, 'Peace' which is one of the parents of 'Super Star' belongs to the Kaempferol type. There can be little doubt that the

Arisumi procedure, if consciously followed, could lead to a great acceleration in the production of roses in which pelargonidin co-exists with the carotenoid type of pigment.

3. The third important proposition which follows from Arisumi's work is whether it is possible to produce a better red rose, based on paeonidin and not cyanidin.

This is again referred to by Dr. Pal in his book. Arisumi's work shows that apart from the cyaniding pigment there are a number of rose species and a few varieties which contain the modified cyaniding type of anthocyanin, paeonin. He points out paeonin produces a red much more brilliant and much less prone to fade, than cyaniding. As referred to earlier, there are a large number of rose species, including *R. foetida bicolor*, *R. rugosa* etc., which contain paeonin. *R. stellata* in fact contains paeonin as the sole anthocyanidin. The Floribunda varieties, 'Piccolo' (Tantau, 1957), 'Red Pinocchio' (Boerner, 1947) and 'Ruby Lips' (Swim, 1958) also contain paeonidin; Arisumi points out that the latter two contain also the genes for synthesizing 3-monoside. There can be little doubt that if the rose breeder is able to combine the genes for the production of paeonidin and that for the formation of 3-monoside in a rose of typical H.T. form he would have taken a giant step forward in producing the perfect red rose.

4. The fourth proposition which flows from Arisumi's work is the question: How do we produce a blue rose?

As readers would be aware, basically a blue rose would be most obviously possible only if the genes synthesizing the anthocyanidin, Delphinidin, were to be introduced in modern roses. Even though Arisumi established as far back as 1968 that delphinidin was in fact present in certain roses, chiefly in the young leaves of the Floribunda 'Samba' (Kordes, 1964), this fact has largely remained unnoticed. I have only seen a passing reference to this sensational finding in an article on the subject of 'Colours in Roses' in the 1980 Rose Annual of the Royal National Rose Society (by Roberts and Humphrey).

But generally, most rose literature till this day dismisses - incorrectly - the fact of delphinidin being present in the gamut of rose species and varieties, and talks only of other and far less obvious procedures for evolving a blue rose. Arisumi's painstaking analysis of the pigment composition of the young leaves of 'Samba' showed beyond doubt that delphinidin was present, though in small quantities. ('Samba' leaves contain 15% delphinidin and 80% cyaniding, the balance being other anthocyanidins). Arisumi has pointed out in his paper that the dream of

the blue rose could be realized if, through selective hybridisation, the delphinidin concentration could be so increased, that it appears in the petals also. From the chemistry point of view, pelargonidin, cyanidin and delphinidin represent a series of anthocyanidins – pelargonidin having a single hydroxyl (OH) group, cyaniding two, and delphinidin, three. Arisumi says conditions are more favourable for hydroxylation in the leaves rather than on rose petals. As such it is easier to have pelargonidin in the rose petal and delphinidin in the rose leaf. But he has pointed out that the position is not quite as hopeless as it may seem – the pigment concentration in the leaf and that in the flower petal are in parallel proportion, and once we achieve a higher concentration of delphinidin in the leaf, this pigment will certainly appear in the petals also.

Arisumi also remarks that existing 'blue' roses are really lavender coloured. These colours are the result of a process technically called 'co-pigmentation' of the red cyaniding pigments and this is what has happened in all lilac or lavender roses, starting from 'Grey Pearl' (1943) and up to 'Paradise' (1979). If this feature of co-pigmentation could also be simultaneously introduced along with a high percentage of delphinidin, Arisumi predicts that the dream of a blue rose would definitely be realized. Readers may well ask 'Where does 'Samba' derive its delphinidin constituent from? Which is the specie responsible?' Unfortunately the parentage of 'Samba' is not given. But a close scrutiny of the Arisumi papers shows that the same characteristic absorption spectrum, which led him to the conclusion that delphinidin was present, is there in the rose species, *R. roxburghii hirtula*. The genes of *R. roxburghii* appear in a host of modern varieties, such as 'Floradora' (Tantau, 1944), 'Traumland' (Tantau, 1958), 'Montezuma' (Swim, 1955), 'Cinnabar' (Tantau, 1945), 'Queen Elizabeth' (Lammerts, 1954) and many others. It is in this welter of rose varieties hopefully containing that most invaluable gene that the rose breeder must struggle if at all a blue rose, based on delphinidin, is to be realized, by traditional methods of cross pollination. And may such a breeder live long enough to attain his goal!

I am sure all rosarians and rose breeders would agree that the researches of Dr. Arisumi deserve their most earnest attention. I may have stretched my neck out by some of my speculations, but the main object in writing this article, apart from bringing to notice the monumental nature of the work done by that great Japanese scientist, would still have been achieved (even if my speculations prove correct), if others are sufficiently interested to try and ascertain the truth, and publicize it widely for the benefit of rose breeders.

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—M. S. VITRARAGHAVAN, Kodaikanal

During the period 1964—1968, the eminent Japanese biochemist, Dr. Kanichi Arisumi, published a series of four papers entitled "Studies in the Flower Colours in Rosa" with special reference to biochemical and genetic analysis, and the application of these results to practical breeding. These papers which represent a masterly synthesis of the subtleties of biochemistry and their application to rose breeding have remained relatively unknown to rosarians, though their content and importance is such that they deserve to be a part of their essential knowledge. This article is an attempt to present from a layman's point of view the main features of these papers, their significance to practical rose-breeding and some further speculations on the theme.

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1. PELARGONIDIN — present in orange red to scarlet flowers e.g. scarlet pelargonium (geranium), orange red dahlia or vermillion roses like SUPER STAR.
2. CYANIDIN — present in crimson to bluish red flowers, e.g. deep red dahlia, red roses such as PAPA MEILLAND.
3. DELPHINIDIN — present in violet to blue flowers, such as delphinium.

Apart from these three basic anthocyanidins there are certain other derivatives which are responsible for modified flower colour: in other words, various shades of red, vermillion, and blue. A number of other factors also influence the colour of anthocyanidins, e.g. iron salts intensify the colour; the pH of the cell sap, presence of tannins, organic acids and enzymes also play a part.

Other than anthocyanidins, there are a number of different pigments, such as FLAVONOLS (e.g. the colouring matter of the pale yellow Sea Rose, Mme. Falcot), and Carotenoid pigments (the colouring matter in carrots, or dark yellow roses), which are responsible for the white and yellow to brown range of flower colours. These pigments may be co-existent along with anthocyanidins with a consequent modification of the pure anthocyanidin colour range. It should also be noted that in many flowers the colour changes with the age of the bloom, e.g. the colour change from yellow to red of the rose variety CHARLESTON, and this is due to the modification of pigment composition during the ageing process.

In the light of the above, we may analyse the colours of roses as follows: Prior to 1900 there were only red (including pink), white and light yellow roses. The red and pink roses, e.g. the Hybrid Perpetuals, owed their colours to the presence of cyanidin; while the pale yellow colours, e.g. of the Tea Roses, were due to the presence of various types of flavonols. Initially the Hybrid Tea roses produced by crossing the Hybrid Perpetuals and the Teas had also the same colour range. But in the early years of the century, the famous French hybridizer, Pernet Ducher, succeeded in crossing the then existing rose varieties into the bright yellow rose species, *R. Foetida* (Persian Yellow); thereby the colour range of roses was greatly increased, because Pernet Ducher's cross brought in the genes for the production of the carotinoid type of pigment. All the bright yellow roses of today ultimately owe their colour to the original cross made by him.

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The analysis of pigments in garden roses showed a remarkable difference in the anthocyanidin constitution in Hybrid Teas and Floribundas, even though both are derived from the common ancestor species, *R. Chinensis*. While the Floribundas had retained the original *Chinensis* i.e. tea rose genes more or less intact, the Hybrid Teas, though they are the result of crosses between Hybrid Perpetuals and Tea roses, had largely the genetic composition of the Hybrid Perpetuals — in other words, that of the tetraploid ancestral types such as Damask and Gallica roses, natives generally of the continent of Europe. As readers may have noted, one of the most interesting features of the *Chinensis* type of rose is the colour changing characteristics — originally of *R. Chinensis mutabilis* and of which there are numerous examples in the Floribunda class, starting from MASQUERADE and including CHARLESTON and many others.

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million coloured Floribundas than H.T.'s, but on the other hand, very few yellow Floribundas. In fact, till today, the yellow floribunda is a comparatively weak class.

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But even more important than the results achieved so far is the guideline provided by Arisumi's work on how to achieve such a result. He points out that pelargonidin tends to co-exist with the Kaempferol type of yellow rose but is not normally found with the Quercetin kind. As mentioned earlier, Kaempferol and Quercetin are flavonols present in a number of rose species and varieties and generally found in most yellow roses in combination with the carotenoid type of pigment. Arisumi showed that while roses of the type of McGREDY'S YELLOW (1933) belong to the kaempferol type, others like LYDIA (Robinson 1949) belong firmly to the Quercetin category. Crosses between McGREDY'S YELLOW and various vermillion roses carrying the pelargonidin gene resulted in a high proportion of seedlings containing pelargonidin. Conversely, similar crosses with LYDIA led to the elimination of pelargonidin in the resultant seedlings.

Apart from the typical Quercetin and Kaempferol type of yellow roses there are others which fall into an intermediate category. Arisumi refers to the variety E. O. BALDWIN (Robinson 1952) in this connection. Here again his work is a little dated by now. But it is easy enough to deduce which of the present day yellow roses belong to the Quercetin or Kaempferol type, by referring to the parentage of modern vermillion roses. Clearly PEACE which is one of the parents of SUPER

STAR belongs to the Kaempferol type. There can be little doubt that the Arisumi procedure, if consciously followed, could lead to a great acceleration in the production of roses in which delphinidin co-exists with the carotenoid type of pigment.

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4. The fourth proposition which flows from Arisumi's work is the question : How do we produce a blue rose?

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But generally, most rose literature till this day dismisses—incorrectly—the fact of delphinidin being present in the gamut of rose species and varieties, and talks only of other and far less obvious procedures for evolving a blue rose. Arisumi's painstaking analysis of the pigment composition of the young leaves of SAMBA showed beyond doubt that delphinidin was present, though in small quantities. (SAMBA leaves contain 15% delphinidin and 80% cyanidin the balance being

other anthocyanidins). Arisumi has pointed out in his paper that the dream of the blue rose could be realized if, through selective hybridization, the delphinidin concentration could be so increased, that it appears in the petals also. From the chemistry point of view, pelargonidin, cyanidin, and delphinidin represent a series of anthocyanidine—pelargonidine having a single hydroxyl (OH) group, cyanidin two, and delphinidin three. Arisumi says that conditions are more favourable for hydroxylation in the leaves rather than on rose petals. As such it is easier to have pelargonidin in the rose petal, and delphinidin in the rose leaf. But he has pointed out that the position is not quite as hopeless as may seem—the pigment concentration in the leaf and that in the flower petal are in parallel proportion, and once we achieve a higher concentration of delphinidin in the leaf, this pigment will certainly appear in the petals also.

Arisumi also remarks that existing 'blue' roses are really lavender coloured. These colours are the result of a process technically called 'co-pigmentation' of the red cyanidin pigments and this is what has happened in all lilac or lavender roses, starting from GREY PEARL (1943) and upto PARADISE (1979). If this feature of co-pigmentation could also be simultaneously introduced along with a high percentage of delphinidin, Arisumi predicts that the dream of a blue rose would definitely be realized. Readers may well ask, Where does SAMBA derive its delphinidin constituent from? Which is the specie responsible? Unfortunately, the parentage of SAMBA is not given. But a close scrutiny of the Arisumi papers shows that the same characteristic absorption spectrum, which led him to the conclusion that delphinidin was present, is there in the rose species, *R. Roxburghi* Hirtula. The genes of *R. Roxburghi* appear in a host of modern varieties, such as FLORADORA (Tantau, 1944), TRAUMLAND (Tantau, 1958), MONTEZUMA (Swim, 1955), CINNABAR (Tantau, 1945), QUEEN ELIZABETH (Lammerts, 1954), and many others. It is in this welter of rose varieties hopefully containing that most invaluable gene that the rose breeder must struggle if at all a blue rose, based on delphinidin, is to be realized, by traditional methods of cross pollination. And may such a breeder live long enough to attain his goal!

I am sure all rosarians and rose breeders would agree that the researches of Dr. Arisumi deserve their most earnest attention. I may have stretched my neck out by some of my speculations, but the main object in writing this article, apart from bringing to notice the monumental nature of the work done by that great Japanese scientist, would still have been achieved (even if my speculations prove incorrect), if others

are sufficiently interested to try and ascertain the truth, and publicize it widely for the benefit of rose breeders.